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Physicochemical and nutraceutical characterization of sirimbache fruit (*Gaultheria glomerata* (Cav.) Sleumer)

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Abstract

The consumption of vegetables with the greatest nutraceutical potential, especially those with high levels of antioxidants such as anthocyanin and phenolic compounds, has become popular among health conscious consumers. The aim of this research was to determine the nutraceutical potential of sirimbache fruits (*Gaultheria glomerata* (Cav.) Sleumer). Characterization of the berries resulted in the following: 11.4 °Brix, 3.25 pH, 0.35% acidity, 83.74% moisture, 0.16% ash, monomeric anthocyanins (112.88 mg cyanidin 3-glucoside / 100 g of sample), total phenolic compounds (344.37 mg of gallic acid / 100 g of sample) and vitamin C (13.87 mg ascorbic acid/ 100 g of sample). These values indicate that sirimbache is a nutraceutical food of great interest. Therefore, the expansion of sirimbache to health conscious markets may be feasible. Such expansion is of great importance for rural agribusiness, as it could promote the development of new agroindustrial projects in the province of Celendín and other Andean areas of Peru.

Keywords: sirimbache; anthocyanins; phenolic compounds; berries; nutraceuticals.

1. Introduction

Nutraceuticals foods have received great attention in the last few years, especially those with a high content of antioxidants, which have been linked to reductions in cardiovascular disease, cancer, neuro-degenerative diseases, inflammation, aging and problems caused by the presence of free radicals (Lobo *et al.*, 2010; Rahman, 2007). Many studies have linked the incidence of different types of cancer to the lower intake of antioxidants (Pham-Huy *et al.*, 2008). Antioxidants act as an electron donor, playing an important role in neutralizing free radicals by ending electron theft chain reactions (Ki and Hyong, 2006). Although several types of antioxidants exist, including anthocyanins and other phenolic

compounds, those present in fruits and vegetables have received the most attention (Pandey and Rizvi, 2009; Tsao, 2010).

Berries are considered nutraceutical foods because they are an important source of phytochemicals, among which are phenolic compounds (flavonoids, anthocyanins, phenolic acids and stilbenes). There are also reports that corroborate the biological activity of these phenols, including: antioxidant activity, anti-inflammatory and anticancer properties (Habauzit and Morand, 2012).

Natural phenolic compounds are secondary metabolites and are an important class of antioxidants found in all plants, with high concentrations often found in vegetables and fruits. An important role of phenolic

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compounds is to protect organisms against oxidative stress induced by free radical species (Cho *et al.*, 2004).

Anthocyanins constitute an important part of phenolic compounds and are responsible for the colors of many fruits and flowers observed in nature (Oh *et al.*, 2008). They are typically found in plant products such as purple corn, purple cabbage, red grape, mulberry, elderberry, purple sweet-corn, blueberry, and strawberry among others (Skrovankova *et al.*, 2015). Various investigations have focused on the health benefits of these pigments, primarily a result of their antioxidant activity (Camire *et al.*, 2002; Hou, 2003). Anthocyanins and anthocyanin extracts from plant material can provide various health benefits, including DNA protection (Lazzé *et al.*, 2003; Ramirez-Tortosa *et al.*, 2001).

In Peru, there is a great variety of fruits, largely unknown to external markets, that can serve as a source of antioxidants (Repo and Encina, 2008). These native fruits are important sources of nutrients, such as vitamins (vitamin C and beta carotene) and minerals (phosphorus and potassium) (Repo and Encina, 2008). Among these is the sirimbache fruit, which is the popular name of the species *Gaultheria Glomerata* in the Celendin province (Cajamarca, norther of Peru). Cultivation of sirimbache has developed extensively with the shrubs used as fences of the cultivated lands, with the berries are marketed in various presentations at craft fairs and local markets. The Experimental Vocational Training Center (CEFOP) located in the Celendin province is one of the few in Peru that has developed agroindustrial products with marketing possibilities based on sirimbache. Based on the excellent results from the work conducted at CEFOP, it is possible that the crop will increase cultivation and application due to new entrepreneurship. For this reason, this work aims to provide a physicochemical characterization of the sirimbache fruit, as well as to determine the content of compounds with important nutraceutical potential, mainly anthocyanins, total phenolic and vitamin C.

2. Material and methods

Plant Material

Sirimbache, originating from Celendin province, Department of Cajamarca, Northern of Peru, was harvested by group or researcher in summer of 2016. The scientific denomination (figure 1) was made for Herbarium Truxillense (HUT) from Universidad Nacional de Trujillo as it follows:

- Popular name: Sirimbache
- Family: *Ericaceae*
- Scientific name: *Gaultheria glomerata* (Cav.) Sleumer

Berries were selected according to ripeness and size and then washed with potable water to separate the foreign materials and dust residues as well as berries subject to mechanical damage. After washing, the Sirimbache fruits were dehydrated and analyzed.

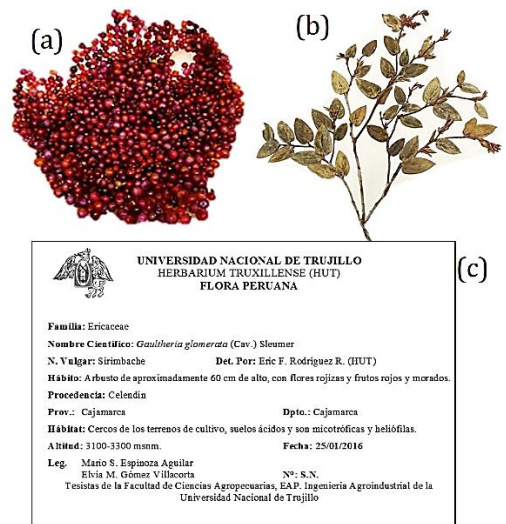


Figure 1. The Sirimbache: (a) Fruits. (b) Bush. (c) Identification file -HUT.

Physicochemical analysis

Soluble solids and pH

Soluble solids were determined using a digital refractometer (PAL-1 BLT /i, Atago, USA) at 20 °C, with the results expressed as Brix degrees. The pH was determined by potentiometric measurement using a pH meter (HI 2210, Hanna Instruments, Spain) according to IFU (2001) methods.

Titrateable acidity and ripeness index

The acidity of the samples was measured using 10.0 g of homogenized fruit diluted in 250 mL of distilled water, and then filtered. Aliquots of 50 mL were transferred to Erlenmeyer flasks and then titrated using 0.1 N NaOH to a pH endpoint of 8.10 as given by a phenolphthalein (1%) indicator, and confirmed with a pH meter (HI 2210, Hanna Instruments, Spain).

The total acidity was expressed as mg citric acid / g sample, and was calculated using the expression: $\text{Acidity} = [(\text{NaOH} \times V_1 \times \text{EQ acid}) / (V_2)]$, where V_1 is the volume of NaOH, V_2 is the volume of the sample, and EQ is the equivalent of the citric acid (64 mg/equivalent) (Cerdán-Calero *et al.*, 2013).

The ripeness index was calculated as the average of the soluble solids in relation to the acid ($^{\circ}\text{Brix}/\text{acidity}$) (Amorós *et al.*, 2003; Pinillos *et al.*, 2011).

Moisture content and ash

The moisture content of the samples was determined by drying 10 g of sirimbache at 105 °C (AOAC, 2000) in an oven (UNB 400, Memmert, Germany) until finding insignificant changes for 3 hours.

The ash was determined by incinerating approximately 3 g of Sirimbache berries at 550 °C - 600 °C inside a muffle furnace (MF 7, Fravill, Peru) for 2 hours.

Analysis of nutraceutical compounds

Total monomeric anthocyanins (TMA)

10 g of crushed Sirimbache was added to 40 mL of alcohol-water solutions (80:20 v/v). The extract was kept cool overnight and filtered the next day using S & S 520 (Schleicher & Schuell - Whatman filter paper grade 520, Maidstone, United Kingdom) (Wicklund *et al.*, 2005).

The TMA was quantified using a pH-differential method according to Giusti's method (Giusti and Wrolstad, 2001); using hydrochloric acid / potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). Briefly, 0.2 mL of the filtrate was mixed with 1.8 mL of one of the buffer solutions and the absorbance of each dilution was measured

at $\lambda = 515$ nm and $\lambda = 700$ nm. A UNICO® 4802 UV-vis double-beam spectrophotometer was used to measure absorption spectra of the system.

The absorbance (A) of the diluted samples was calculated using the following formula: $A = (A_{515} - A_{700}) \text{pH } 1.0 - (A_{515} - A_{700}) \text{pH } 4.5$ (1)

Where A is the absorbance of the diluted samples. A_{515} is the absorbance measured at λ_{515} , and A_{700} is the measured absorbance at λ_{700} .

The total monomeric anthocyanins content was expressed as cyanidin-3-O-glucoside per g of fruit and was calculated by:

$$\text{TMA} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(A \cdot M_W \times \text{DF} \times 100)}{M_A \cdot L} \quad (2)$$

Where:

M_W is the molecular weight (449.2 g/mol); DF is the dilution factor (50); M_A is the extinction coefficient 26.900 L/ (cm* μmol); L = path length (1cm).

Total phenolics compounds

Total phenols were determined according to (Espinoza *et al.*, 2016; Holtung *et al.*, 2011; Viuda-Martos *et al.*, 2010) with some modifications. The analyses were performed by visible spectrophotometry (4802 UV-vis, UNICO®, USA) after reaction with the Folin-Ciocalteu reagent (Merck Millipore®, Darmstadt-Germany). Ethyl alcohol (80° v/v) was added to 2.0 g of crushed sirimbache and filled up to 10 mL in a centrifuge tube. This mixture was stirred and centrifuged at 4200 rpm for 15 minutes then filtered using S & S 520 (Schleicher & Schuell - Whatman filter paper grade 520, Maidstone, United Kingdom).

The extracts from the samples (20 μL) were added to test tubes with 1580 μL of distilled water and 100 μL of Folin-Ciocalteu reagent. This mixture was kept in the dark at room temperature for 20 minutes and then transferred into a 40 °C water bath (WNB 14, Memmert, Germany) with 300 μL addition of 20% sodium carbonate solution (w/v) for 15 min.

The absorbance of all samples was measured at 760 nm after incubation at 50

°C for 10 minutes in an oven (UNB 400, Memmert, Germany). The results were calculated using a calibration curve obtained from a standard of gallic acid (Sigma-Aldrich®, Spain) and expressed as mg of gallic acid / 100 g of sirimbache.

Content of ascorbic acid

The ascorbic acid content was assessed by the 2, 6-dichloroindophenol titrimetric method, according to Varming *et al.* (2013). Briefly, 5.0 g of crushed fruit was diluted with 50 mL of oxalic acid solution (1 g/100 mL) (Merck, Darmstadt, Germany) and then filtered using S & S 520 (Schleicher & Schuell - Whatman filter paper grade 520, Maidstone, United Kingdom) and transferred to a volumetric flask. After preparing the samples, aliquots of 10 mL were transferred to Erlenmeyer flasks, a further 50 mL of oxalic acid solution was added, and the solution was then mixed and titrated with 2, 6-dichloroindophenol solution (0.02 g/100 mL) (Sigma-Aldrich®, Denmark). The final point was considered when the solution had a pink color for 15 s. The calibration of the 2, 6-dichlorophenolindophenol solution was performed with 0.05% ascorbic acid solution (Merck, Darmstadt, Germany) and expressed as milligrams of ascorbic acid / 100 g of sirimbache. All quantifications were conducted in triplicate.

3. Results and discussion

Sirimbache fruit's characterization

Physicochemical analysis

The values of the initial parameters, as well as the physicochemical and functional characteristics of the sirimbache fruit, are shown in Table 1. A comparison of data from sirimbache and other berries previously studied is shown in Table 2, in order to provide perspective on the physicochemical and functional characteristics of better-studied berries in relation to sirimbache.

The pulp of the sirimbache had a soluble solids content between 10 and 15 °Brix, which is equivalent to values of other berries as shown in Table 2. The pH of the pulp is consistent with previous studies, which found pH values between 3.0 and 3.4 for wild fruits (Arteaga and Arteaga, 2016). In addition, these values are consistent with those of other berries, including blackberries and strawberries where pH of 3.23 and 3.4, respectively were found (Hassimotto *et al.*, 2008). Because the main organic acid present in the genus *Vaccinium* is citric acid (Kalt and McDonald, 1996), the acidity of the Sirimbache was expressed as citric acid, in order to simplify comparison with the other fruits.

Table 1

The physicochemical and functional composition of Sirimbache (g/100 g of fruit)

Composition	Sirimbache	
	w.b X±S	d.b X±S
Soluble solids ^(a)	11.43 ± 0.15	ND
pH	3.25 ± 0.05	NDV
Titrateable acidity ^(b)	0.346 ± 0.005	NDV
Ripeness index ^(c)	33.08 ± 0.44	NDV
Moisture content (%)	83.74 ± 0.03	NDV
Ash (%)	0.162 ± 0.001	0.996 ± 0.006
Total monomeric anthocyanins ^(d)	112.88 ± 0.72	694.20 ± 0.09
Total phenolics ^(e)	344.37 ± 0.06	2117.91 ± 0.03
Vitamin C ^(f)	13.87 ± 0.14	85.20 ± 0.83

w.b: wet basis; d.b: dry basis; X: average; S: standard deviation. NDV: No determinate value.

^(a) °Brix at 20 °C.

^(b) mg citric acid/g of sample.

^(c) Ratio °Brix/titrateable acidity.

^(d) mg cyanidin 3-glucoside / 100 g sample.

^(e) mg gallic acid / 100 g sample.

^(f) mg of ascorbic acid / 100 g sample.

Table 2

Comparison of sirimbache composition with other berries

Composition	Sirimbache	Cranberry	Blackberry (Andean)	Strawberry	Goldenberry	Blackberry (Chester)
Soluble solids ^(a)	11.43	---	9.00 ⁽⁵⁾	11.06 ⁽¹¹⁾	14.06 ⁽¹⁵⁾	12.25 ⁽¹⁹⁾
pH	3.25	3.2 ⁽²⁾	2.88 ⁽⁶⁾	3.48 ⁽¹¹⁾	3.58 ⁽¹⁶⁾	3.23 ⁽¹⁹⁾
Titrateable acidity ^(b)	0.346	0.86 ⁽²⁾	0.87 ⁽⁷⁾	0.93 ⁽¹¹⁾	1.59 ⁽¹⁶⁾	0.93 ⁽²⁰⁾
Moisture content (%)	83.74	87.3 ⁽¹⁾	88.76 ⁽⁶⁾	89.3 ⁽¹²⁾	81.3 ⁽¹⁷⁾	82.98 ⁽²⁰⁾
Ash (%)	0.16	0.21 ⁽³⁾	0.4 ⁽⁸⁾	0.69 ⁽¹³⁾	1.17 ⁽¹⁶⁾	0.42 ⁽²⁰⁾
Total monomeric anthocyanins ^(d)	112.88	259.5 ⁽⁴⁾	270 ⁽⁹⁾	450.10 ⁽¹¹⁾	---	109.07 ⁽²⁰⁾
Total phenols ^(e)	344.37	519.5 ⁽⁴⁾	118 ⁽⁹⁾	147.80 ⁽¹⁴⁾	58.6 ⁽¹⁶⁾	400.67 ⁽²⁰⁾
Vitamin C ^(f)	13.87	13 ⁽³⁾	10 ⁽¹⁰⁾	0.27 ⁽¹³⁾	43.3 ⁽¹⁸⁾	14.37 ⁽²⁰⁾

^(a) °Brix a 20 °C.^(b) mg citric acid/g of sample.^(c) Ratio °Brix / titrateable acidity.^(d) mg cyanidin 3-glucoside / 100 g sample.^(e) mg gallic acid / 100 g sample.^(f) mg of ascorbic acid / 100 g sample.⁽¹⁾ Garrido and Pérez-Urria (2014).⁽²⁾ Sapers *et al.* (1984).⁽³⁾ Prior *et al.* (1998).⁽⁴⁾ Moyer *et al.* (2002).⁽⁵⁾ Tosun *et al.* (2008).⁽⁶⁾ Ayala *et al.* (2013).⁽⁷⁾ Kopjar *et al.* (2009).⁽⁸⁾ Carmona *et al.* (1996).⁽⁹⁾ Kuskoski *et al.* (2004).⁽¹⁰⁾ Velazco and Vega (2003).⁽¹¹⁾ Yorgey *et al.* (1995).⁽¹²⁾ Sellappan *et al.* (2002).⁽¹³⁾ Martínez-Soto *et al.* (2008).⁽¹⁴⁾ Sun *et al.* (2002).⁽¹⁵⁾ Rossi *et al.* (2012).⁽¹⁶⁾ Guevara-Pérez and Málaga-Barreda (2013).⁽¹⁷⁾ Yildiz *et al.* (2015).⁽¹⁸⁾ Repo and Encina (2008).⁽¹⁹⁾ Hassimotto *et al.* (2008).⁽²⁰⁾ Valencia and Guevara-Pérez (2013).

As shown in Table 2, the measured moisture content of the berries examined here is relatively narrow and ranges from 81.3% to 83.74% for Goldenberry and sirimbache, respectively. The ash content of sirimbache was found to be $0.162 \pm 0.001\%$ on the wet basis, values very close to cranberry. In addition, other studies suggest that the ash content of sirimbache ranked between 0.15 to 0.31% (Hassimotto *et al.*, 2008) which agrees with the value found for sirimbache (0.162%).

Nutraceutical compounds analysis

Total monomeric anthocyanins

Anthocyanins are the most important group of water-soluble pigments in plants. More than 550 unique anthocyanin compounds have been reported, which is distributed mainly in flowers, pulp and peel fruits and vegetables (Kong *et al.*, 2003; Skrovankova *et al.*, 2015). Anthocyanins are also responsible for the orange, red, purple and blue colors. Their chemical proportions depend on many factors such as the pH of the medium, temperature, among others (Peña, 2006). The total monomeric anthocyanins value was 112.88 ± 0.72 mg cyanidin 3-glucoside / 100 g of fresh extract. This value is similar to blackberries (Hassimotto *et al.*, 2008), but substantially lower than values for other berries (Table 2).

Total phenolics

Phenolic compounds can play several roles as antioxidants, and also have key sensory attributes (bitterness and astringency), which is why they have attracted the attention of many researchers (Hamauzu *et al.*, 2006). The total phenol content in Sirimbache was 344.37 ± 0.06 mg gallic acid / 100 g fresh weight, more than 154 mg gallic acid equivalent / 100 g (w.b) greater than that for Goldenberries (Repo and Encina, 2008), indicating that the sirimbache fruit has an important content of phenolic compounds. Many authors have identified that the primary causes of differences in anthocyanin and total phenol content between different species of berries are factors such as surface to volume ratio of the species, area of cultivation, environmental conditions before harvesting, genetic differences, differences in maturation and different degrees of ripeness at the time of harvest (Taruscio *et al.*, 2004; Zadernowski *et al.*, 2005). Differences in phenolic compounds could also be related to different handling procedures during postharvest storage (Kalt and McDonald, 1996).

Vitamin C

Is a potent antioxidant. Ascorbic acid has the ability to remove different reactive oxygen species, maintain α -tocopherol in

the reduced state, act as a cofactor by maintaining the activity of a number of enzymes (by keeping the metal ions in the reduced state). Evidence also suggests that it can serve as a substrate for oxalate and tartrate biosynthesis, and has a role in stress resistance (Klein and Kurilich, 2000). Sirimbache was found to contain 13.87 ± 0.14 mg of ascorbic acid / 100 g sample. This value is substantially lower than has been identified for goldenberries, 43.3 ± 0.5 mg ascorbic acid / 100 g (Repo and Encina, 2008), but is still comparable to *Chester* variety blackberries, 14.3 mg ascorbic acid / 100 g sample; *Andean* variety blackberries, 10 mg ascorbic acid / 100 g sample (Pantelidis *et al.*, 2007; Velazco and Vega, 2003); and cranberries.

4. Conclusions

The physicochemical composition on a wet basis of sirimbache fruits was examined. The content of nutraceutical compounds in sirimbache fruit was also determined. These results indicate that sirimbache is largely comparable to a variety of berries, including blackberries, strawberries, and goldenberries. From this, sirimbache fruit can be considered a functional food of a great interest for the food agro-industry due to its high content of total monomeric anthocyanins, phenolics and vitamin C. Because of this, additional projects to promote the development of sirimbache as an agro-industrial crop are warranted.

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